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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/82, C07K 14/47, A01H 5/00, G01N 33/68, C12Q 1/68

A3

(11) International Publication Number: **WO 00/26391**

(43) International Publication Date: 11 May 2000 (11.05.00)

(21) International Application Number: PCT/US99/25522

(22) International Filing Date: 29 October 1999 (29.10.99)

(30) Priority Data:

60/106,321	30 October 1998 (30.10.98)	US
60/138,303	9 June 1999 (09.06.99)	US

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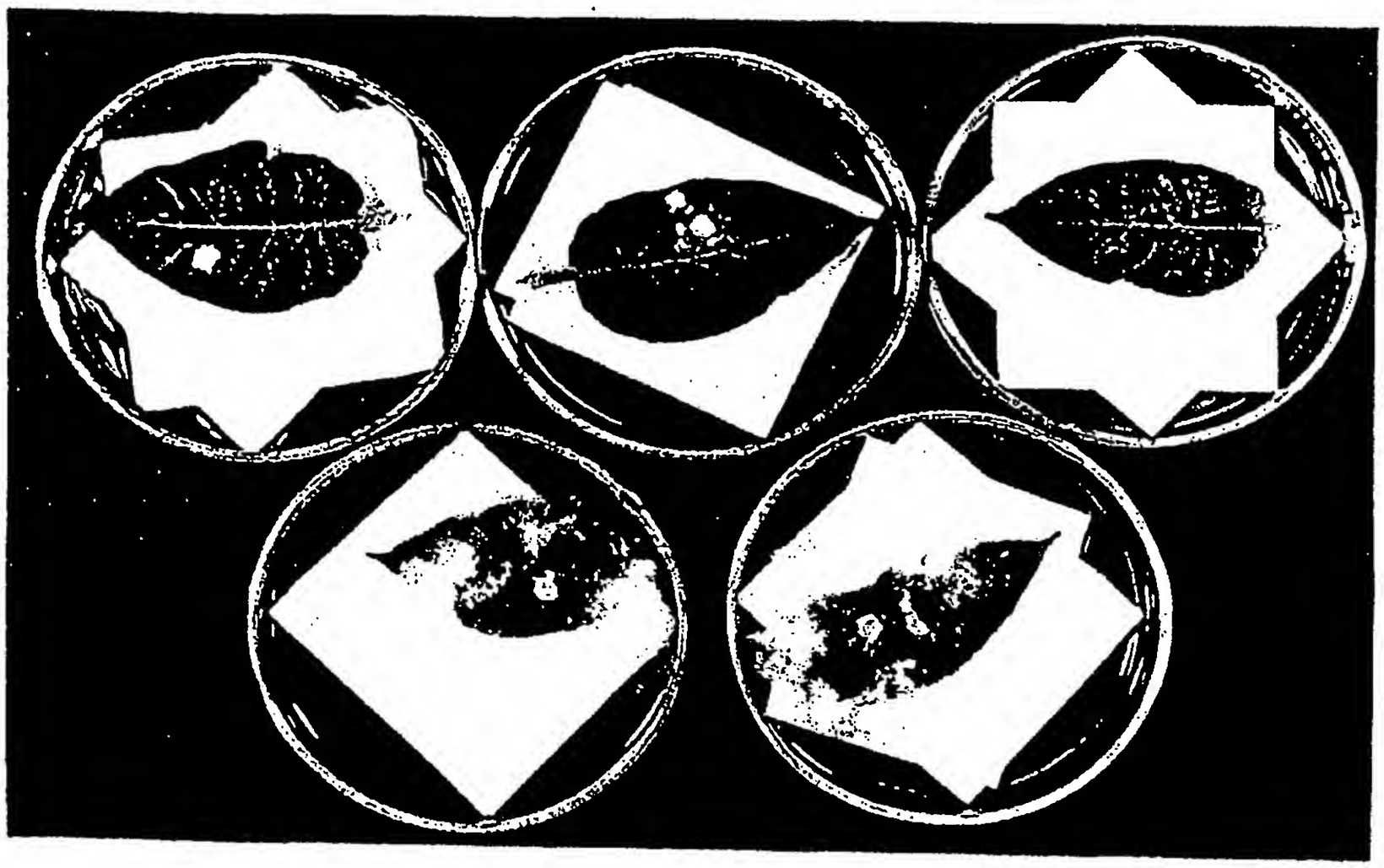
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
26 October 2000 (26.10.00)

(54) Title: TRANS–SPECIES TRANSFER OF APOPTOTIC GENES AND TRANSGENIC PLANTS DEVELOPED THEREBY



(57) Abstract

The invention relates to trans–species transfer of apoptotic genes to plant cells, transgenic plants developed therefrom, and screening assays using these plants. The invention also relates to drug discovery screening methods utilizing transgenic plant cells. In addition, the invention relates to methods of identifying plant apoptotic pathway components utilizing non–plant proteins and nucleic acids.

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 99/25522

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C07K14/47 A01H5/00 G01N33/68 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 864 650 A (DIRECTOR GENERAL OF NATIONAL I) 16 September 1998 (1998-09-16) the whole document --- -/--	1-6, 10-14, 24-32, 36-40, 50-53, 57-59, 63,64, 66-71,79

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 2000	20.07.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 99/25522

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	MITTLER ET AL: "Inhibition of programmed cell death in tobacco plants during a pathogen-induced hypersensitive response at low oxygen pressures" PLANT CELL,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 8, no. 11, November 1996 (1996-11), pages 1991-2001-2001, XP002106912 ISSN: 1040-4651 the whole document	1-4,6, 10,11, 13,15, 17-20, 27-30, 32,36, 37, 39-41, 43-46, 53,54, 56,59, 60,63, 64, 66-71,79
X	WO 96 35703 A (HUMAN GENOME SCIENCES INC ;HE WEI WU (US); ROSEN CRAIG A (US); HUD) 14 November 1996 (1996-11-14) the whole document	1-4,10, 11,13, 14, 27-30, 36,37, 39,40, 59, 61-64, 67-71,79
A	WO 96 34956 A (ARCH DEV CORP ;US GOVERNMENT (US)) 7 November 1996 (1996-11-07) the whole document	
A	WO 97 35971 A (ADAMS JERRY MCKEE ;HOLMGREEN SHAUN P (AU); CORY SUZANNE (AU); GIBS) 2 October 1997 (1997-10-02) the whole document	
A	WO 98 13493 A (UMANSKY SAMUIL ;MELKONYAN HOVSEP (RU); LXR BIOTECHNOLOGY INC (US)) 2 April 1998 (1998-04-02) the whole document	
P,X	WO 98 54961 A (NOVARTIS ERFINDUNGEN VERWALTUN ;NOVARTIS AG (CH)) 10 December 1998 (1998-12-10) page 3; example 8	1-4,6,8, 13,14, 27-30, 32,34, 39,40, 53-56, 71,79

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 99/25522

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 09664 A (GILCHRIST DAVID G ;RICHAEL CRAIG (US); LINCOLN JAMES E (US); UNIV) 24 February 2000 (2000-02-24) the whole document	1-3,6, 10,11, 13-15, 17,18, 21, 27-29, 32,36, 37, 39-41, 43,44, 47,54, 56,59, 60,63, 64, 66-71,79

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INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 99/25522

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-3,4,5,6-11,13-29,30,31,32-37,39-54,55,56,57,58,59-71,79 (inventions 1,2,3,4)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3,6-11,13-29,32-37,39-54,56,57,59-71,
79 completely, 4,5,12,30,31,38,55,58 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein Ced-9; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene and methods to generate such plants, methods to modulate apoptosis in plants by transformation with such a gene.

2. Claims: 4,5,30,31,55,58 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein Bcl-2; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

3. Claims: 4,30,55,58 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein Bcl-xL; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

4. Claims: 4,30,55,58 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein IAP; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

5. Claims: 4,30,55,58 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein E1B 19k; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

6. Claims: 12,38 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein caspase; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. Claims: 12,38 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein rev-caspase; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

8. Claims: 12,38 partially

A transgenic plant expressing a heterologous gene encoding other members of the Bcl-2 family not covered by inventions 1,2,3,10,11; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

9. Claims: 12,38 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein Apaf-1; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

10. Claims: 12,38 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein Bad; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

11. Claims: 12,38 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein Bax; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

12. Claims: 12,38 partially

A transgenic plant expressing a heterologous gene encoding the apoptotic pathway protein ced-4; said plant being rendered resistant to biotic and abiotic insults; furthermore a plant cell comprising said gene.

13. Claims: 72,73 completely

Method to identify plant specific genes having apoptotic pathway activity by expressing a plant cDNA library in animal cells and screening for apoptotic activity.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

14. Claims: 74-78 completely

Method to identify heterologous apoptotic genes that function in plants by expressing a heterologous cDNA library in plants and screening for apoptotic activity in said plants.

INTERNATIONAL SEARCH REPORT

...information on patent family members

Inte onal Application No
PCT/US 99/25522

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0864650 A	16-09-1998	JP 10309142 A	24-11-1998
		AU 703009 B	11-03-1999
		AU 5832398 A	17-09-1998
		CA 2231738 A	11-09-1998
		CN 1195026 A	07-10-1998
		JP 2000023583 A	25-01-2000
WO 9635703 A	14-11-1996	CA 2220670 A	14-11-1996
		AU 2548095 A	29-11-1996
		EP 0830375 A	25-03-1998
		JP 11507208 T	29-06-1999
WO 9634956 A	07-11-1996	AU 5673496 A	21-11-1996
		CA 2220012 A	07-11-1996
		EP 0826043 A	04-03-1998
		JP 11504814 T	11-05-1999
WO 9735971 A	02-10-1997	AU 2143997 A	17-10-1997
		CA 2250207 A	02-10-1997
		EP 0932674 A	04-08-1999
WO 9813493 A	02-04-1998	AU 4651397 A	17-04-1998
		EP 0932678 A	04-08-1999
WO 9854961 A	10-12-1998	AU 8535598 A	21-12-1998
		EP 0986299 A	22-03-2000
		ZA 9804681 A	02-12-1998
WO 0009664 A	24-02-2000	AU 5772599 A	06-03-2000